It only remains for me to record my great indebtedness to Dr. S. G. Platts for the assistance which he has rendered me throughout a considerable part of this investigation.

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The Influence of the Hydrogen Concentration upon the Optimum Temperature of a Ferment.

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The present investigation is an outcome of previous work,[*] resulting in the discovery that the optimum temperature of any ferment, or ferment function, occurring in a given enzymic preparation, is independent of the concentration both of the substrate and of the enzyme, the duration of the action being constant. To follow up this observation it was felt desirable to investigate in a similar way the influence, if any, of the reaction, that is of the hydrogen ion concentration of the medium, on the optimum temperature of enzyme action: the more so because enzymes, as regards their activity, are known to be extremely sensitive to this factor—some requiring an acid, others a neutral, and others again an alkaline medium in which to act. The question, moreover, is of special interest on account of the fact that Sörensen, in his classical researches on the *rôle* of the ionic concentration of the medium in activating enzymes, alludes to it, and predicts in regard to it that, when investigated, the optimum temperature will no doubt be found to vary with the hydrogen ion concentration of the medium.[†] That opinion, being of a speculative nature, was not deemed a sufficient answer to the question; its experimental investigation therefore became the more needed.

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[*] Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 87, p. 245 (1914); B, vol. 88, p. 258 (1914).

[†] S. P. L. Sörensen, 'Comptes Rendus des Travaux du Laboratoire de Carlsberg,' vol. 8, p. 148 (1909).

The enzyme chosen was the *maltase* of *Aspergillus oryzæ*, the same preparation being used as had already been studied in a former communication,[*] where its optimum temperature, in an action of 16 hours' duration, and H^+ concentration that of the preparation simply dissolved in redistilled water, is shown to be +47°.

Two stages occur in the investigation: (1) A determination of the optimum reaction curve of the enzyme in an action of 16 hours' duration at +47°, for chosen dilutions of substrate and of enzyme, in presence of progressively increasing quantities of acid and of alkali added to the reaction mixture; (2) separate determinations of the optimum temperature of the ferment under the same conditions of substrate concentration, of enzyme concentration, and of duration of the experiment, for different hydrogen ion concentrations of the medium, corresponding to various points on the above optimum reaction curve.

The substrate concentration chosen was M/20, or 18×10^{-3} grm. of hydrated maltose per cubic centimetre of the reaction mixture; and the enzyme concentration was 6×10^{-4} grm. of the enzyme preparation in powder per cubic centimetre of the reaction mixture.

For the determination of the optimum reaction curve the practical details were as follows:—First, a solution of the enzyme was prepared by dissolving 30 mgrm. of the preparation in 10 cm.^{3} of redistilled water. A clear pale amber-coloured solution resulted, which, after standing from a half to one hour at the ordinary temperature, was introduced in portions of 1 cm.^{3} into a series of eight clean test-tubes (Fischer's extra resistance glass) already containing 90 mgrm. of hydrated maltose and either 4 cm.^{3} of pure water or a solution containing a known quantity of acid or alkali. The tubes, after closing with clean sterile corks, were plunged into a water bath regulated at +47°. After 16 hours' incubation they were withdrawn, the corks removed, and each rapidly washed with 1 cm.^{3} of water—the washings being carefully added to the contents of the corresponding tube. Next, the tubes were heated for seven minutes in boiling water to stop the enzyme action, after which they were cooled and the contents diluted to 50 cm.^{3}. The proportion of maltose hydrolysed was then determined, by Bertrand's method,[†] on 20 cm.^{3} of the diluted mixture. The numbers obtained are set out in Table I.

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[*] *Ibid.*

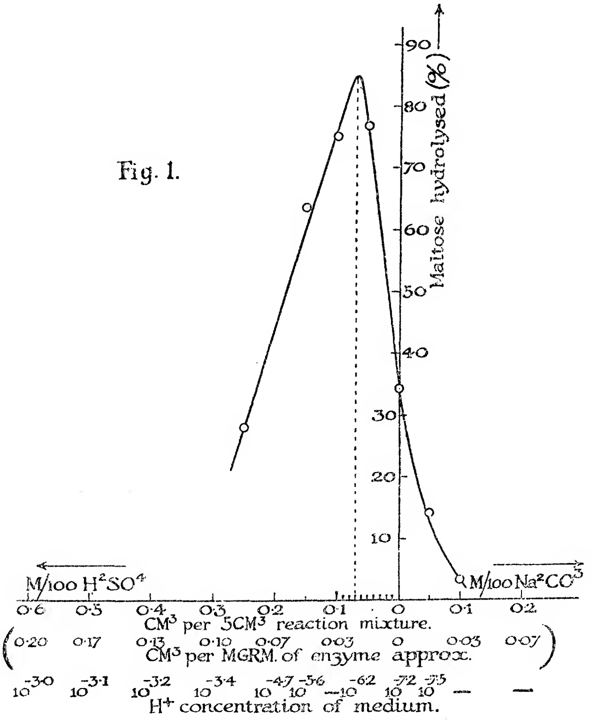
[†] 'Bull. Soc. Chim.,' (3), vol. 35, p. 1285 (1906).

Table I.

Quantities of acid and alkali added per 5 cm.^{3} of the reaction mixture.	Maltose hydrolysed.
	Per cent.
0·25 cm.^{3} M/100 H_2SO_4	28·2
0·15 „ „	63·5
0·10 „ „	75·1
0·05 „ „	76·8
0·00 „ (natural reaction ; control)	34·3
0·05 „ M/100 Na_2CO_3	14·2
0·10 „ „	3·2

When the percentage of maltose hydrolysed is plotted against the quantities of reagents added, the curve indicated in fig. 1 is obtained.

Fig. 1.



This curve shows that under the conditions of the experiment the addition of the merest trace of alkali is detrimental to the enzyme action ; while on the contrary the addition of acid increases the efficiency of the enzyme, until a certain point is reached, corresponding to the presence of 0·07 cm.^{3} M/100 H_2SO_4 in the 5 cm.^{3} reaction mixture, beyond which the addition of more acid is in turn detrimental to the action. In other words, the action passes by a maximum situated in the acid region.

Measurement of the hydrogen ion concentration of the medium, resulting from the addition of various quantities of acid and of alkali per 5 cm.^{3} of the reaction mixture, containing 3 mgrm. of dissolved enzyme, were made by the colorimetric method of Sörensen (*loc. cit.*). The results are contained in Table II, and for convenience of description, in what follows, they have been reproduced on the base line of fig. 1, underneath the respective quantities of M/100 H_2SO_4 and M/100 Na_2CO_3 which give rise to them.

Table II.

Quantities of acid and of alkali added per 3 mgrm. of enzyme contained in 5 cm.^{3} of the reaction mixture.		Corresponding H^+ concentrations.
0·60 M/100 H_2SO_4	H^+ concentrations greater than the optimum of fig. 1.	$10^{-3.0}$; citrate, methyl orange.
0·40 ,, ...		$10^{-3.2}$; ,, ,,
0·30 ,, ...		$10^{-3.4}$; ,, ,,
0·20 ,, ...		$10^{-4.7}$; phosphate, neutral red.
0·15 ,, ...		$10^{-5.6}$; ,, ,,
0·07 ,, ...	H^+ concentrations equal to the optimum of fig. 1.	$10^{-6.2}$; ,, ,,
0·04 ,, ...	H^+ concentrations less than the optimum of fig. 1.	$10^{-6.8}$; ,, ,,
0·00 (natural reaction)		$10^{-7.2}$; ,, ,,
0·05 M/100 Na_2CO_3......		$10^{-7.5}$; ,, ,,

The second stage of the enquiry, which consists in determining the optimum temperature of the enzyme for a series of hydrogen ion concentrations corresponding to different points on the optimum reaction curve, figured in fig. 1, was next undertaken. Nine different H^+ concentrations of the medium were thus studied: $10^{-3.0}$, $10^{-3.2}$, $10^{-3.4}$, $10^{-4.7}$, $10^{-5.6}$, $10^{-6.2}$, $10^{-6.8}$, $10^{-7.2}$, and $10^{-7.5}$.

The practical details of the first determination in the series may be given as an example, to show how these optimum temperature determinations were carried out. A solution of the enzyme was prepared containing 3 mgrm. of the preparation per cubic centimetre and, after standing for a half to one hour, was introduced in portions of 1 cm.^{3} into eight clean test-tubes, already containing 90 mgrm. of maltose, 0·6 cm.^{3} of M/100 H_2SO_4 and 3·4 cm.^{3} of redistilled water. Such a mixture, according to Table II (or fig. 1), corresponds to a H^+ concentration of $10^{-3.0}$. The tubes were closed with sterile corks, plunged into water thermostats at known temperatures, and incubated for 16 hours, when the enzyme action was stopped and the quantity of maltose hydrolysed in each tube determined as before. The numbers found are set out in Table III, together with the numbers obtained for the other members of the series.

Table III.

Temperatures at the beginning and end of each experiment.	Maltose hydrolysed per cent. for the following H^+ concentrations of the medium.								
	$10^{-3\cdot0}$.	$10^{-3\cdot2}$.	$10^{-3\cdot4}$.	$10^{-4\cdot7}$.	$10^{-5\cdot6}$.	$10^{-6\cdot2}$.	$10^{-6\cdot8}$.	$10^{-7\cdot2}$.	$10^{-7\cdot5}$.
17 ·6–17 ·7°	—	—	—	—	21 ·1	—	—	—	3 ·2
17 ·7–17 ·8	11 ·4	10 ·0	—	—	—	—	—	—	—
18 ·0	—	—	15 ·5	—	—	—	—	—	—
23 ·0	—	—	—	—	—	26 ·8	—	7 ·2	—
24 ·5–24 ·6	—	—	24 ·0	—	—	—	—	—	—
24 ·8–25 ·0	—	—	—	28 ·2	29 ·7	—	—	—	8 ·6
25 ·0–25 ·1	—	21 ·1	—	—	—	—	16 ·9	—	—
25 ·2–25 ·4	21 ·1	—	—	—	—	—	—	—	—
27 ·8–27 ·9	—	—	—	—	—	35 ·9	—	12 ·8	—
32 ·0–31 ·6	—	—	—	40 ·6	—	—	—	—	—
32 ·2–32 ·1	28 ·2	—	—	—	—	—	—	—	—
32 ·8	—	35 ·9	39 ·1	—	52 ·5	—	32 ·8	—	12 ·8
34 ·5–34 ·6	—	—	—	—	—	57 ·1	—	21 ·1	—
37 ·8–38 ·2	28 ·2	—	—	—	—	—	—	—	—
40 ·0	19 ·7	39 ·1	50 ·9	—	71 ·7	—	52 ·5	—	15 ·5
41 ·8	—	—	—	—	—	78 ·5	—	28 ·2	—
42 ·3–42 ·2	11 ·4	—	—	—	—	—	—	—	—
42 ·4–42 ·5	—	28 ·2	—	—	—	—	—	—	—
42 ·5–42 ·6	—	—	—	63 ·5	—	—	—	—	—
45 ·4	—	—	—	—	76 ·8	—	—	—	21 ·1
46 ·8–47 ·0	—	8 ·6	18 ·3	—	—	—	—	—	—
47 ·0–45 ·6	—	—	—	—	—	—	66 ·8	—	—
47 ·0	—	—	—	46 ·4	—	85 ·5	—	32 ·8	—
49 ·7–49 ·6	—	—	—	—	—	83 ·8	—	28 ·2	—
49 ·8–49 ·6	—	—	—	—	—	—	66 ·8	—	—
49 ·9–49 ·8	—	—	7 ·2	—	43 ·5	—	—	—	12 ·8
50 ·3	—	—	—	16 ·9	—	—	—	—	—
52 ·0	—	—	—	—	31 ·2	66 ·8	49 ·4	21 ·1	—
52 ·1–51 ·9	—	—	—	—	—	—	—	—	10 ·0
53 ·9–53 ·8	—	—	—	—	—	—	39 ·1	—	—
54 ·0	—	—	—	—	16 ·9	49 ·4	—	15 ·5	5 ·9

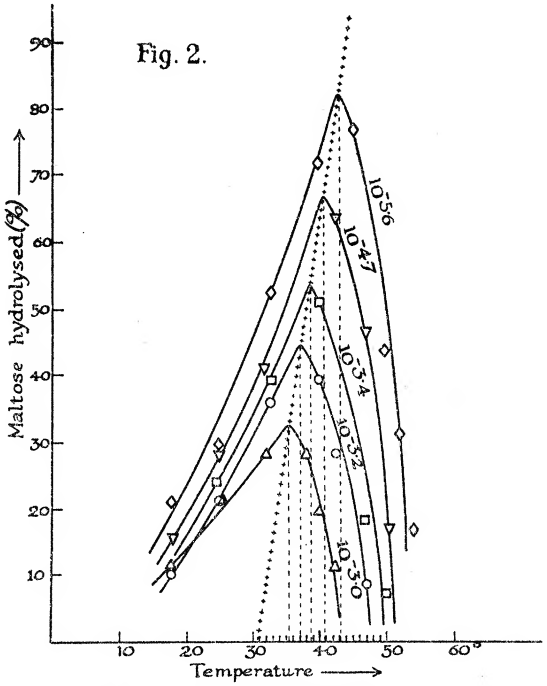
On plotting the percentage of maltose hydrolysed against the mean temperature of the experiment, these numbers give a series of optimum temperature curves from which the optimum temperature corresponding to each particular H^+ concentration of the medium may be read. The curves are summarised for purposes of description in two figures (figs. 2 and 3). In fig. 2 are collected the curves corresponding to H^+ concentrations greater than the optimum reaction of fig. 1; while in fig. 3 are collected the curves corresponding to H^+ concentrations equal to, and less than, the optimum of fig. 1.

Consider fig. 2. Here we have a series of curves of varying altitudes, which is what one would expect from the results already recorded in fig. 1; as the H^+ concentration of the medium is increased beyond the optimum value the enzyme is gradually rendered less efficient. And, in this respect, fig. 2 indicates, further, that the diminution in the activity is true for practically all temperatures, although varying in amount from temperature to temperature, subject to the influence—to be explained presently—of the hydrogen ion concentration of the medium.

As to the influence, if any, of the H^+ concentration of the medium on the optimum temperature of the enzyme, fig. 2 clearly shows that for each H^+ concentration there exists a perfectly definite optimum temperature; also, the optimum temperature is seen to fall progressively as the H^+ concentration is increased beyond the optimum reaction of fig. 1. In fact, the locus of the maxima of these curves is a straight line, the mathematical equation of which is approximately

$$y = 6{\cdot}56x - 200{\cdot}44.$$

Fig. 2.



H^+ concentrations varying between 10^{-3} & $10^{-5.6}$

The significance of this straight line is that it shows that the fall which occurs in the optimum temperature of the ferment as the H^+ concentration is increased—beyond its optimum value—is proportional to the fall in activity (or disablement) which the ferment undergoes at the physical optimum point.

Fig. 2 further shows that the temperature of destruction of the enzyme also depends on the hydrogen ion concentration of the medium; the greater the acidity the less heat is the enzyme able to support before being entirely disabled. Herein lies the explanation of the existence of the two lower curves of fig. 2, in spite of the indications of fig. 1, which shows that for the H^+ concentrations of $10^{-3\cdot2}$ and $10^{-3\cdot0}$ no activity on the part of the enzyme seems possible. The reason is, that what was the optimum temperature for the natural reaction, +47°—at which temperature the results set forth in fig. 1 were determined—is no longer the optimum temperature for the H^+ concentrations giving rise to the curves in question, but is instead a temperature of destruction.

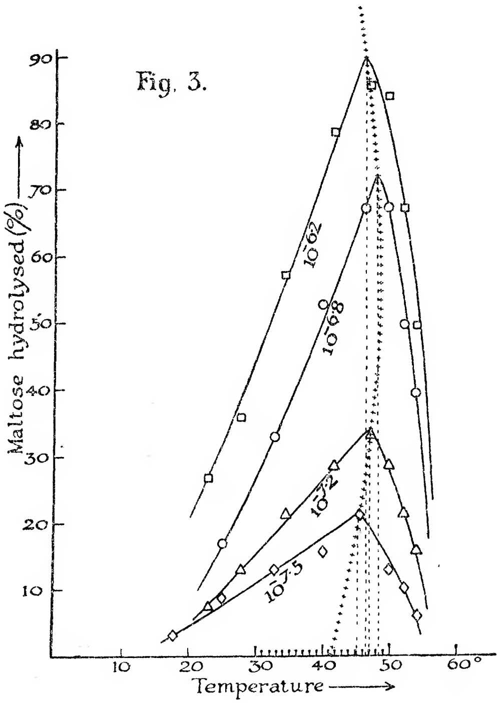


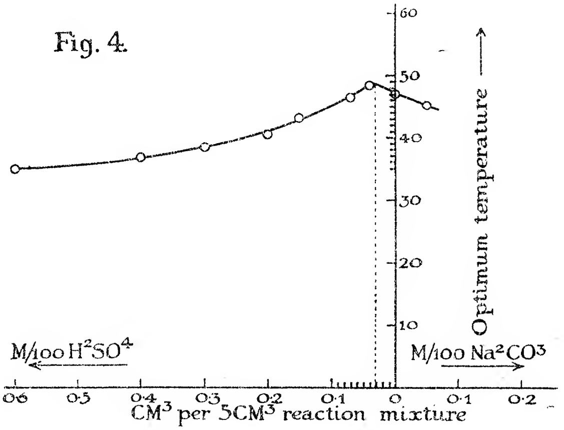
Fig. 3.

H^+ concentrations varying between $10^{-6.2}$ & $10^{-7.5}$

That the optimum temperature of the ferment diminishes as the H^+ concentration is increased beyond that of the chemical optimum may be stated otherwise thus: As the H^+ concentration is diminished from extreme values to values bordering on that of the chemical optimum, the optimum temperature steadily increases. An interesting question now arises. What would be the effect on the optimum temperature of diminishing the H^+ concentration beyond that of the chemical optimum? Would the optimum temperature under these circumstances continue to increase, in view of the fact that further diminution of the H^+ concentration of the medium must, in accordance with fig. 1, be attended by disablement of the enzyme? Fig. 3 answers these questions.

Fig. 3, as expected, also shows a series of curves of varying altitudes; and, as before, a perfectly definite optimum temperature is seen to characterise each hydrogen ion concentration studied. But here an unlooked-for result is discerned. For H^+ concentrations situated between the optimum and the natural reaction the optimum temperature rises still higher, to fall again as the natural reaction is overreached; the rise in the optimum temperature passes by an optimum value. This is evident from the locus curve drawn through the maxima of the several optimum temperature curves figured in fig. 3; and it is still better seen in fig. 4, where a curve is plotted with the various optimum temperatures recorded in figs. 2 and 3 as ordinates, and the corresponding amounts of acid and alkali present in the reaction mixture as abscissæ.

Fig. 4.



An optimum temperature of +49° thus seems possible in a reaction mixture containing 0·032 cm.^{3} M/100 H_2SO_4. But it might be supposed that this is the optimum reaction of the ferment for that temperature, since, in accordance with the work of O'Sullivan and Tompson* on the enzyme *sucrase,* the optimum reaction of that ferment is known to depend on the temperature serving for its determination? This question it is proposed to investigate.

To illustrate in a striking way the essential fact established by the investigation, that the optimum temperature of the enzyme depends largely on the hydrogen ion concentration of the medium, it is only necessary to reproduce side by side on the same diagram the optimum temperature curve from fig. 3 corresponding to the natural reaction and that from fig. 2

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* 'Chem. Soc. Journ.,' vol. 57, p. 859 (1890).

corresponding to the addition to the reaction mixture of 0·2 cm.^{3} M/100 H_2SO_4 per milligramme of enzyme. This is done in fig. 5.

Fig. 5.

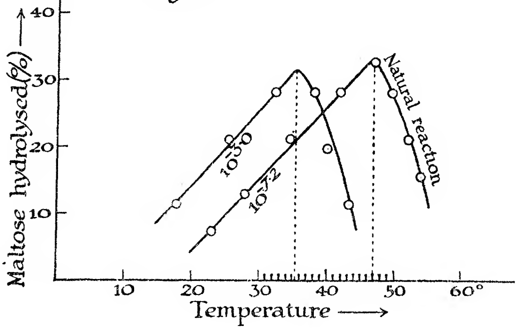

Fig. 5 shows at a glance that by the simple process of increasing the hydrogen ion concentration of the medium in which the enzyme acts from $10^{-7\cdot2}$ to 10^{-3} it is possible to change the optimum temperature of the ferment from +47° to +35·5°,—*i.e.* through a range of 11·5°. This result alone shows how important it is, when stating the optimum temperature of an enzyme, to point out at the same time the H^+ concentration of the medium serving to determine it. Thus, the optimum temperature of the *maltase* in question, in an action of 16 hours' duration, may be expressed as $[35\cdot5°]\,10^{-3\cdot0}$ or $[47°]\,10^{-7\cdot2}$.

Fig. 5 is further interesting from another aspect. The parallelism or similarity of the two curves is very striking; they are almost superposable. Were they exactly so, and obviously that is only a question of sufficient experimental patience, it would mean that the activity of the enzyme is the same at corresponding temperatures over two equal although different ranges of temperature,—the one curve being in that case simply a translation in the plane of the paper of the other. It is proposed to term this, the phenomenon of *corresponding states.* From a consideration of the locus lines of figs. 2 and 3, it is evident that for H^+ concentrations such that the maxima of the resulting optimum temperature curves are situated at the same level on these lines, the enzyme should for the one and the other H^+ concentration be in "corresponding states."

The main conclusion of the present paper, in the insight which it affords into the influence of the H^+ concentration of the medium on the physical optimum of enzyme action, cannot perhaps be summarised to more advantage at present than by placing it in its appropriate place in a differential table briefly setting out our present knowledge of the relation of the physical and the chemical optima of enzyme action to the experimental conditions involved in determining them.

OPTIMUM TEMPERATURE.	*v.*	OPTIMUM H^+ CONCENTRATION.
(Physical Optimum.)		(Chemical Optimum.)
i. Is independent of the concentration of the enzyme (Compton). *Maltase, salicinase.*		i. Ditto (Sörensen). *Sucrase.*
ii. Is independent of the concentration of the substrate (Compton). *Maltase, salicinase.*		ii. Is dependent on the concentration of the substrate (Van Slyke and Zacharias).[†] *Urease.*
iii. Is dependent on the H^+ concentration of the medium (Compton). *Maltase.*		iii. Is dependent on the temperature of the experiment (O'Sullivan and Tompson). *Sucrase.*
iv. Is dependent on the duration of the experiment serving to determine it (Bertrand and Compton).[*] *Amygdalinase, amygdalase.*		iv. Ditto (Sörensen). *Catalase, pepsin, sucrase.*
		v. Is dependent on the age of the enzymic preparation (Bertrand and Compton).[‡] *Amygdalinase, amygdalase.*

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[*] 'Comptes Rendus,' vol. 152, p. 1518 (1911).

[†] 'Journ. Biol. Chem.,' vol. 19, p. 205 (1914).

[‡] 'Comptes Rendus,' vol. 159, p. 434 (1914).